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SEARCH REQUEST FORM

CRFE

Requester's Full Name: DAVID GUZO Examiner #: 70677 Date: 7/25/05
Port Unit: 1636 Phone Number: 2-0767 Serial Number: 10738454
Location (Bldg/Room#): Remain 2A75 (Mailbox #): 2670 Results Format Preferred (circle): PAPER DISK

To ensure an efficient and quality search, please attach a copy of the cover sheet, claims, and abstract or fill out the following:

Title of Invention: _____

Inventors (please provide full names): _____

Earliest Priority Date: _____

Search Topic:
Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the expected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc., if known.

For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

Please run a regular plus interference sequence search
on SEQ ID NO: 24.

1
747NA

Thanks

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Date Searcher Picked Up: 7/27/05
Date Completed: 8/1/05
Searcher Prep & Review Time: _____
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Type of Search
____/____ NA Sequence (#)
____/____ AA Sequence (#)
____/____ Structure (#)
____/____ Bibliographic
____/____ Litigation
____/____ Fulltext
____/____ Other

Vendors and cost where applicable

____/____ STN ____/____ Dialog
____/____ Questel/Orbit ____/____ Lexis/Nexis
____/____ Westlaw ____/____ WWW/Internet
____/____ In-house sequence systems
____/____ Commercial ____/____ Oligomer ____/____ Score/Length
____/____ Interference ____/____ SPDI ____/____ Encode/Transl
____/____ Other (specify)

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ALIGNMENTS

RESULT 1
AY403874
AY403874
AY403874

344 bp
DMM
11900x
CSC-12-DEC-2002

genomic survey sequence.

VERSION AY403874.1 GI:39759857

SOURCE	Mus musculus (house mouse)
ORGANISM	Mus musculus

REFERENCE	1 (bases 1 to 344)
AUTHORS	Clark, A. G., & Glanville

Todd, M.A., Tanenbaum, D.M.,
Fertiera, S., Wang, G., Zhe

TITLE Inferring nonneutral evolution from human-chimp-mouse orthologous

gene trials
Journal of Science 302 (5652), 1960-1963 (2003)

FORMED	146 / 1302
REFERENCE	2 (bases 1 to 344)

Todd, M.A., Tanenbaum, D.M., Civello, D.R., Lu, F., Murphy, B.,

Adams, M.D. and Cargill, M.

JOURNAL Submitted (16-NOV-2003) Celera Genomics, 45 West Gude Drive,

COMMENT This sequence was made by sequencing genomic exons and ordering

FEATURES	Location/Qualifiers
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/organism="Mus musculus"
/mol_type="Genomic DNA"
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/db_xref="taxon:100
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/locus_tag="HCML702"

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Matches 280;  Conservative  0;  Mismatches  3;  Indels  0;  Gaps  0;

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Db 58 GAGGCTGCAGTCACCCAAGCCCAAGAAACAAGGTGGCAGTAA CAGGAGGAAGGTGACA 117

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PD	22-JUL-1999.	
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PF	20-JAN-1999;	99WC-US001188.
XX		
PR	20-JAN-1998;	98US-00009388.
PR	26-AUG-1998;	98US-00140084.
XX		
PA	(UNII) UNIV ILLINOIS FOUND.	
XX		
PI	Wittnup KD, Kleke MC, Kranz DM, Shusta B, Roder ET,	
XX		
IR	WPI, 1999-430619/36.	

GenCore version 5.1.6
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OM nucleic - nucleic search, using sw model

Run on: July 29, 2005, 06:57:21 ; Search time 3600 Seconds

(without alignments) 10054.457 Million cell updates/sec

Title: US-10-738-454-24

Perfect score: 747

Sequence: 1 gacgtcgagtcgacccaag.....ttgtctacacatcatctag 747

Scoring table: IDENTITY_NUC

Gapop 10.0 , Gapext 1.0

Searched: 4708233 seqs, 24227607955 residues

Total number of hits satisfying chosen parameters: 9416466

Minimum DB seq length: 0

Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%

Listing first 45 summaries

Database : GenEmbl:*
1: gb_ba:*
2: gb_hg:*
3: gb_in:*
4: gb_om:*
5: gb_ov:*
6: gb_pat:*
7: gb_ph:*
8: gb_pl:*
9: gb_pt:*
10: gb_ro:*
11: gb_scs:*
12: gb_sy:*
13: gb_un:*
14: gb_vl:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	Query Match	Length	DB ID	Description
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3	747	100.0	747	6 AR476748	Sequence
4	747	100.0	747	6 AR478145	Sequence
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6	342.2	45.8	1372	6 I02550	Sequence
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9	294.8	39.5	435	10 MMTCRAB	Sequence
10	283	37.9	852	12 SCUS9428	Sequence
11	281	37.6	2625	6 AR362567	Sequence
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ALIGNMENTS

RESULT 1	AR220110	Sequence 24 from patent US 6423538.	DNA	linear	PAT 26-SEP-2002
LOCUS	AR220110				
DEFINITION	Sequence 24 from patent US 6423538.				
ACCESSION	AR220110				
VERSION	AR220110.1	GI:23324541			
KEYWORDS	Unknown.				
SOURCE	Unknown.				
ORGANISM	Unknown.				
REFERENCE	Unclassified.				
AUTHORS	Wittrop, K.D., Kranz, D.M., Keike, M. and Boder, E.T.				
TITLE	Yeast cell surface display of proteins and uses thereof				
JOURNAL	Patent: US 6423538-A 24 23-JUL-2002;				
FEATURES	Location/Qualifiers				
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DB	241 GCTACCCCTGCACTGATGATGATGATGATGATGATGATGATGATGATGATGATG	300			

Institute of Medicine, Humboldt University, Berlin, Medical Dept of the

variable region of the ly CB41 and a detailed by peptide mutations led model of the antigen-antibody interaction shows two hydrophobic a salt bridge (L:Arg50) argy. In order to verify the model and expressed a scFv-antibody (scFv41, KD = 100 kDa) with important residues by site directed mutagenesis. H:Tyr32Ala scFv lacking no binding activity. The scFv with out the salt bridge (L:Arg50) KD. These results seem to confirm, first crystallographic L3-loop conformation in

essig, S., Hausdorf, G., Sauer, H., Giessmann, E., and Schomburg, D. (1993)

mutant of antibody D1.3 increased binding to hen egg

F.P. Schwarz^{a,b}, R.J. Pohlmann^a, *Research in Biotechnology, of Chemical Engineering, 01, USA.*

selection by phage display of which bind with improved affinity. Interestingly, no residues on the surface were altered in the side chains can influence binding such a way as to improve binding is reported to bind HEL

change upon HEL binding of the mutant M3, using the method of Freire et al. [2] we find that $\Delta\Delta H$ and $\Delta\Delta C_p$ type. $\Delta\Delta G$ will be determined by surface plasmon resonance quench titrations. Preliminary stabilization achieved by favourable configurational changes to binding free energy hydrophobic effect will be the method of Freire et al. [2] of mutant antibodies with the help guide the in vitro providing structural insights enhanced binding.

[1] Hawkins et al. (1993) *J. Mol. Biol.* 234, 958.

[2] Freire et al. (1995) *Proteins* 21, 83.

Isolation and characterization of human single chain Fv (scFv) against botulinum neurotoxin type A.

Peter Amersdorfer^a, Cindy Wong^a, Theresa Smith^b, James D. Marks^a, *^aDepartment of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, CA 94110, ^bToxinology Division, USAMRIID, Frederick MD 21702, USA.*

Botulinum neurotoxins, which cause the flaccid paralysis associated with the disease botulism, are proteins composed of two polypeptide chains. The light chain possesses the enzymatic activity and the heavy chain is responsible for binding to neuronal membranes. The carboxy-terminal half of the heavy chain (H_C) mediates neurospecific binding and the amino-terminal half of the heavy chain (H_N) assists in internalization of the toxin. To produce human antibodies capable of toxin neutralization, a human immune scFv phage antibody library was generated using peripheral blood lymphocytes of an individual immunized with botulinum penta-valent toxoid (ABCDE). The V_H and V_L genes were amplified by PCR and spliced together to create an scFv gene repertoire which was cloned into pCANTAB 5E (Pharmacia) to create a library of 7.7×10^5 members. Hybridisation of the unselected library using V_L or V_H light chain specific primers demonstrated 66% V_H light chain genes and 33% V_L light chain genes. The library was selected on botulinum toxin type A (BTA) immobilized on polystyrene and after 4 rounds of panning, 84 out of 92 clones bound the toxin. Nucleotide sequencing revealed that 26 different scFv antibodies have been isolated. Native scFv were expressed to determine their specificity by ELISA on BTA, recombinant BTA translocation domain and C-fragment domain, 15 of these bound BTA, but not H_C or H_N . Four scFv bound both BTA and H_N . Seven scFv bound both BTA and H_C . Binding studies of scFv against the H_C , which is believed to play a key role in neutralization of the toxin, will be presented.

Antibodies as modulators of protein activities — implications for intravenous therapy.

Kerstin Andersson, Ulla-Britt Hansson, *Dept. of Biochemistry, Chemical Center, P.O. Box 124, S-221 00 Lund, Sweden.*

We have examined the modulating effects of non-immune human IgG on the activities of biospecific molecules like antibodies and enzymes.

Non-immune human IgG was found to inhibit the binding of antigen by specific antibodies of both human and rabbit origin. Human immunoglobulins were also able to modify the composition of preformed antigen-antibody complexes. Furthermore, the presence of non-immune human IgG was found to affect the activity of enzymes (yeast glucose-6-phosphate dehydrogenase and human placental alkaline phosphatase). We have also observed some odd interactions of immunoglobulins with antibodies used as affinity ligands.

None of the observed effects could be explained only as a result of activities of specific antibodies in the non-immune IgG preparations. Taken together, our results suggest that

immunoglobulins may interact with each other and with other proteins not only as antibodies against antigens, but also through interactions which are distinct from antigen-binding.

A network of such 'non-immunological' interactions would be of great importance in providing suitable conditions for physiological protein activities and may, at least in part, explain the beneficial effects of intravenous therapy in autoimmune conditions. It is also easy to conceive a regulatory function of immunoglobulins similar to the allosteric regulation of, for instance, enzymatic activities through this kind of interactions.

Yeast surface display system for antibody engineering.

Eric T. Boder, K. Dane Wittrup, *Department of Chemical Engineering, University of Illinois-Urbana-Champaign, Urbana, Illinois 61801, USA.*

Progress in antibody engineering has been largely stimulated by methodological advances. Phage display technology is a powerful and popular means to generate new antibodies and to mature existing antibodies for improved affinity or specificity through successive rounds of mutagenesis and selection by 'panning'. This technique requires expression of the antibody library in *E. coli*, a host organism which exhibits a strong expression bias against many heterologous proteins. Conversely, the protein processing and secretory machinery of the yeast *Saccharomyces cerevisiae* bears striking homology to that of mammalian cells, while the organism remains easily manipulable through molecular genetics techniques. Thus, yeast is an ideal choice for the expression of libraries of antibodies or other mammalian proteins for the purpose of directed evolution.

A surface display system for the *in vitro* expression and selection of peptide and protein libraries on yeast has been developed. A nine residue peptide epitope (HA) has been fused to the binding subunit of a yeast cell wall protein (AGA2), followed by the 4-4-20 anti-fluorescein single-chain F_v . Selection was performed by fluorescence activated cell sorting (FACS). Single-pass and double-pass enrichment factors have been determined by FACS performed on mixtures of cells with and without the displayed fusion. This system presents the potential for the *in vitro* affinity maturation of antibodies as well as the directed evolution of other proteins and peptides, with the advantages of (i) a double-label FACS selection scheme allowing finer affinity discrimination than panning; (ii) as many as 10^4 copies of the displayed sequence per cell, eliminating stochastic variations in the selection; and (iii) library expression in yeast, with an altered or potentially improved expression bias which could yield clones which would be deleted from a library expressed in *E. coli*.

A bacterial surface-expression system using OmpA fusion-proteins.⁴

Timo M. Breit, Ton Logtenberg, *Department of Immunology, University Hospital, Utrecht, the Netherlands.*

⁴ (this work was supported by the Dutch Organisation for Scientific Research).